Detection of phospho-Histone H3 (Ser10) in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagents

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Kit: Rabbit Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog: PK-6101
Note: The Vector Rabbit Elite Kit contains solutions needed to make the block, secondary and label antibodies.

Avidin Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # SP-2001

Primary antibody: Rabbit anti-phospho-Histone H3 (Ser10)
Upstate Cell Signaling Solutions
Lake Placid, NY 12946
www.upstate.com
1-800-233-3991
Catalog # 06-570

Negative control serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: Papilloma Skin (mitotic cells)
Stain Localization: Nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Unmasking technique using the decloaker.
   Add 500 ml distilled water to the pan of the decloaker.
   Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.
   Decloak for 5 minutes. Pressure_____
   Depressurize for 10 minutes.
   Remove pan top and cool for 10 minutes. Temperature before cooling_____
   Rinse in distilled water twice for 3 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

5. Apply blocking solution from the Vector Rabbit Elite Kit and incubate for 20 minutes at room temperature.
   Exp. Date_____ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply the Avidin Biotin Blocking kit
   Lot#_____ Exp Date_____New Kit: yes / no
   Apply avidin block - 15 minutes at room temperature.
   Quick rinse in 1X Automation Buffer.
   Apply biotin block - 15 minutes at room temperature.
   No wash, wipe excess block and apply primary antibody

   DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Histone H3) at a 1:500 dilution and incubate for one hour at room temperature.
   Lot#_____ Exp Date _____

   For negative control slides, normalize the protein concentration of the normal rabbit serum to match the protein concentration of the primary antibody (Histone H3), and use this to make a 1:500 dilution. Apply to slides and incubate for one hour at room temperature.
   Lot#_____ Reconstituted Date _____

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody from the Rabbit Elite Kit and incubate for 30 minutes at room temperature.

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

11. Apply Label antibody from Rabbit Elite Kit and incubate for 30 minutes at room temperature. (Prepare at least 30 mins prior to use)

12. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.
    (Add 1 drop of DAB per ml of substrate)
    Lot#_____ Exp. Date_____ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation buffer until they turn blue.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% Ethanol	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 10/17/06